Manipulating the system: How large herbivores control bottom-up regulation of grasslands

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Abstract
1. Decades of grazing studies have identified a number of key plant and soil processes affected by large herbivores and how those grazer effects vary among different grassland types. However, there remains little mechanistic understanding about how the effects of grazers on plants and soils may be biogeochemically linked in regulating grassland processes.
2. Here we measured monthly plant and soil variables, including soil moisture, soil nitrogen (N) availability, plant biomass, shoot N concentration and plant production, in grazed and ungrazed (fenced) grasslands during the 2012–2014 growing seasons. Measurements were used to assess direct and indirect biogeochemical pathways by which grazers influenced net above-ground plant production (NAP) in dry and mesic grasslands in Yellowstone National Park (YNP).
3. Herbivores only had direct effects on plant variables at the dry grassland compared to direct and indirect effects on both plant and soil variables at the mesic grassland. By enhancing leaf N content at both grasslands, grazers shifted the resource controlling NAP from N in ungrazed grassland to moisture, and potentially phosphorus and/or other soil nutrients, in grazed grassland.
4. Synthesis. These results indicate the mechanistic linkage between top-down (herbivore) and bottom-up (soil resource) control of grassland production. Changing the resources that limit net above-ground plant production (NAP) likely has a profound impact on how grazed vs. ungrazed Yellowstone National Park (YNP) grasslands respond to environmental (e.g., climate, atmospheric N deposition) variability. Because grazing enhances leaf N among many types of grasslands, increasing the sensitivity of plant production to the availability of moisture and nutrients other than N may be a general response of grasslands to grazing.

KEYWORDS
bottom-up regulation, grassland, herbivory, plant production, plant–herbivore interactions, soil moisture, soil nitrogen, top-down regulation, trophic control, Yellowstone National Park

1 | INTRODUCTION

More than a third of the earth’s terrestrial surface is grassland supporting herds of wild and domesticated ungulates (Conant, Paustian, & Elliot, 2001; Follett, Kimble, & Lal, 2001; Lal, 2004) that have profound effects on terrestrial processes. Ungulates have been shown to alter above-ground production by as much as ~30% and +65% (Frank, McNaughton, & Tracy, 1998; Milchunas & Lauenroth, 1993), the rate of...
soil net nitrogen (N) mineralization by −50% and +100% (Bakker, Knops, Milchunas, Ritchie, & Olff, 2009; Frank & Groffman, 1998), and soil carbon (C) concentration by −72% and +146% (Conant et al., 2001; Dermer & Schuman, 2007; McSherry & Ritchie, 2013). The large ranges of grazer effects are a function of several contingent factors (Bardgett & Wardle, 2003; Milchunas & Lauenroth, 1993; Wardle et al., 2004). For example, herbivores generally increase above-ground production under light to moderate grazing regimes, when production is high, and herbivores and vegetation have a long co-evolutionary history. Conversely, herbivory tends to reduce above-ground production when grazing intensity is high, production is low, and vegetation and herbivores have short co-evolutionary histories. However, such phenomenological knowledge of herbivore effects falls short of a mechanistic understanding of how the multiple pathways by which herbivores influence biogeochemical processes may combine to regulate production in grazed grassland.

There are several terrestrial models that simulate grassland biogeochemical processes (Bachelet, Lenihan, Daly, & Neilson, 2000; Boer & Stafford Smith, 2003; Chang et al., 2015; Chen, Lee, Lee, & Oikawa, 2007; Coughenour, 1993, 2005; Henderson et al., 2015; Hidy et al., 2012; Parton, Stewart, & Cole, 1988). Their use in simulating grazed grassland, however, is problematic, because many require parameterization of the functional responses of plants and soils to grazing that are usually not known and none includes all of the multiple pathways that herbivores can influence plant and soil processes. Because grassland soil C is a substantial percentage of the global C pool (Anderson, 1991; Eswaran, van den Berg, & Reich, 1993; Schlesinger & Bernhardt, 1977), by inference, herbivory is likely an important driver of the global C cycle. However, before grazed grassland can be realistically simulated and the future feedbacks between grasslands and climate be accurately forecasted, an improved biogeochemical understanding of herbivore effects on plant and soil processes will be required.

Simulating the effects of herbivory on plant production requires knowledge of the direct and indirect biogeochemical pathways that link grazing to the availability of soil resources (i.e., moisture and nutrients) and ultimately plant growth. Herbivory has been shown to change microclimate (by increasing incident radiation and reducing leaf transpiration surface area and, consequently, moisture loss), grass tillering (which stimulates growth of young physiologically active tissue), leaf N concentration, soil surface light intensity and leaf photosynthetic rates (Coughenour, McNaughton, & Wallace, 1985; Georgiadis, Ruess, McNaughton, & Western, 1989; Knapp et al., 1998; McNaughton, 1984, 1985; McNaughton, Wallace, & Coughenour, 1983; Wallace, 1990). Furthermore, grazers can increase soil N availability (i.e., net N mineralization rate), the most limiting nutrient in temperate grasslands (Fay et al., 2015), by adding urine and dung to the soil that have N in forms more readily available to soil microbes than that in plant litter, and increasing root exudation that stimulates microbial activity in the rhizospheres of grazed plants (Frank & Groffman, 1998; Hamilton & Frank, 2001; Hamilton, Frank, Hinchey, & Murray, 2008; McNaughton, Ruess, & Seagle, 1988). Together, these studies identify a number of different pathways by which herbivores may change resources that control plant production. However, they provide little insight about how herbivore-induced changes in plant and soil processes are propagated along biogeochemical pathways that affect availabilities of soil resources and plant growth.

The goal of this study was to examine the direct and indirect effects of herbivores on Yellowstone National Park (YNP) grassland plant and soil processes. Earlier investigations revealed which ecosystem processes were subject to herbivore control. The aim of this study was to determine how the herbivore effects on different plant and soil processes control the availability of resources (particularly moisture and N) that limit grassland production in YNP. This approach, we hoped, would provide a more comprehensive understanding of how herbivores regulated grasslands than had been previously possible. We focused on controls on net above-ground production (NAP), because of the importance of plant production in supporting grassland food webs, influencing the economies of rural communities around the globe and driving terrestrial CO₂ sink—source dynamics (Follett & Reed, 2010). Our first step was to develop a conceptual model of the various pathways that herbivores influence soil and plant processes, including NAP. We then evaluated the conceptual model with measurements of soil and plant processes in grazed and ungrazed (fenced) grassland to test the underlying mechanisms by which herbivores influence grassland NAP. Because plant and soil properties and the response of grasslands to grazing vary markedly along the topo-edaphic gradient in YNP (Frank, Inouye, Huntly, Minshall, & Anderson, 1994; Frank, Wallen, & White, 2016), we contrasted herbivore control of NAP at a mesic valley-bottom and dry upland grassland.

## 2. MATERIALS AND METHODS

### 2.1. Study sites

The study was performed in grazed grasslands in northern YNP. Two grasslands were examined: a mesic grassland located on the flood plain of the Lamar River in the Lamar Valley and a dry grassland on a broad bench near the confluence of Crystal Creek and the Lamar River. The sites were 3 km apart. Soil at the mesic grassland was deeper and more C- and N-enriched than soil at the dry grassland. Mean (±SE, n = 3) 0–10 cm soil C and N at the mesic grassland were 5.3% (±0.4%) and 0.5% (±0.1%) at and the dry grassland were 3.0% (±0.1%) and 0.3% (±0.1%), respectively. There was greater species richness at the mesic (34) compared to the dry (18) grassland. Dominants (species with >10% relative cover) at the mesic grassland were *Poa pratensis* (25%) and *Trifolium repens* (15%). The dry grassland was dominated by *Poa sandbergii* (44%), *Antennaria microphylla* (14%) and *Stipo comata* (13%).

YNP supports eight species of ungulates. Elk (*Cervus canadensis*), bison (*Bison bison*) and pronghorn (*Antilocapra americana*) are the predominant ungulate grassland grazers in YNP. The park also supports populations of bighorn sheep (*Ovis canadensis*) and mountain goats (*Oreamnos americanus*) that primarily graze cliffs, ridgetops, and steep high elevation slopes, and mule deer (*Odocoileus hemionus*), moose (*Alces alces*), and white-tailed deer (*Odocoileus virginianus*) that rarely graze grassland. The study was conducted in an area occupied during the growing season by large numbers of bison, which was the principal grazing ungulate species at our study sites (Geremia et al., 2014).
Climate of YNP includes long cold winters and short dry summers. Thirty-year (1984–2014) mean annual temperature and precipitation at Tower Falls, located 5 and 7 km from the dry and mesic grassland sites, respectively, were 2.4°C and 40.6 cm. The study was performed during the April–September growing seasons between 2012 and 2014. Growing season (April–Sept) precipitation was lower in 2013 (21.5 cm) compared to 2012 (27.9 cm) and 2014 (31.3 cm).

2.2 Measurements

Monthly rates of ungulate consumption (g shoot removed m⁻² month⁻¹) and NAP (g m⁻² month⁻¹) in grazed grassland were determined with replicated (n = 6) moveable exclosures (Frank, 2007; Frank, Kuns, & Guido, 2002; Frank & McNaughton, 1992). Moveable exclosures were randomly relocated monthly at each grassland during each growing season. Consumption was calculated as the difference between standing plant biomass of a 0.5 m⁻² quadrat in the enclosure and the average biomass in two 0.5 m⁻² quadrats randomly located approximately 3 m from the enclosure. Monthly NAP in grazed grassland was determined as an increment in standing biomass inside each moveable enclosure.

Three exclosures fixed in place 4 weeks after snowmelt were used to measure NAP in ungrazed grassland. A positive increment in standing biomass in a 0.5 m⁻² quadrat inside each fixed exclosure was defined as NAP. Monthly measures of NAP and consumption were converted to daily rates (g m⁻² day⁻¹) to account for the small variation in the length of the sampling intervals. Standing biomass was determined by the canopy intercept method (Frank, 2007; Frank et al., 2002), which allowed us to discriminate between live (green) and senesced tissue.

Soil moisture was measured in two random locations in each sampling quadrat in grazed grassland and fixed exclosures in 2013 and 2014 with the HS2 TDR system (Campbell Scientific). Average soil moisture for each quadrat was determined on paired measurements.

Soil inorganic N availability was measured with 2.5 × 10 cm cation and anion exchange membranes in 2013. Pairs of each type of membrane were inserted vertically into the soil by creating a slit in the soil with a putty knife. Membranes were randomly located within 3 m of each temporary enclosure in grazed grassland and inside each fixed enclosure. For ungrazed grassland in fixed exclosures, membranes were inserted just beyond the outside edges on opposite sides of each quadrat used to measure plant biomass. Soil was gently pressed to close the slit and ensure contact between the soil and the membrane. Membranes were left in the soil during three consecutive periods in 2013: May 28 to June 18; June 19 to August 13; and August 14 to October 2. Cation and anion membranes were impregnated with sulfonic acid and quaternary ammonium, respectively, and both were pretreated with HCl acid and neutralized with NaHCO₃. Membranes were kept cool on ice during shipment to Washington and Lee University and refrigerated until analysis by spectrophotometry. Total ammonium and nitrate adsorbed to membranes was determined on a mg N m⁻² day⁻¹ basis.

Shoot N concentration was determined on days that biomass was estimated in all 3 years in grazed grassland and inside fixed exclosures. Six random samples of shoot material were collected in grazed grassland. In fixed exclosures, samples were collected just outside plant biomass quadrats in a manner that avoided resampling areas during the growing season. Shoot material was rinsed with deionized water, dried and ground to a powder with a Wiley Mill. Shoot N concentration was determined on a CE Elantech Soil Analyser.

We estimated the percent cover of monocots and dicots at peak above-ground biomass in 2013 and 2014 at both grasslands. Percent cover was estimated in a 0.5 m² quadrat inside and two paired quadrats roughly 3 m outside each fixed enclosure.
2.3 | Conceptual model

We developed a conceptual model of the direct and indirect pathways by which herbivores and climate can influence the availability of soil moisture and N (Figure 1), the two principal factors that regulate production of temperate non-calcareous grassland (Fay et al., 2015; Harpole & Tilman, 2007; Hooper & Johnson, 1999; Kirchner, 1977; Parton et al., 1988; Risser, 1985). We hypothesized the following. (1) Soil moisture was determined by recent precipitation, average daily temperature and the amount of standing leaf surface area (associated with community-level transpiration). (2) Shoot biomass was influenced by soil moisture and grazing. (3) Soil N availability was affected by average soil moisture and daily temperature during the sampling interval, and grazing. Grazing affected soil N availability indirectly by changing soil moisture (via altering transpiration rates) and directly by adding dung and urine and stimulating root exudation and rhizospheric microbial activity (Chen et al., 2017; Frank & Groffman, 1998; Hamilton & Frank, 2001; Hamilton et al., 2008). (4) Leaf N concentration was a function of soil moisture, soil N availability, and the direct stimulating effect that grazing had on the production of young N-rich tillers (McNaughton, 1984). Indirect control of herbivores on leaf N concentration included the influence on soil moisture and soil N availability. (5) The monthly rate of NAP was determined by soil moisture, leaf N concentration (closely related to photosynthetic capacity), and the green shoot biomass at the beginning of the sampling period (reflecting the capacity of the vegetation to respond to an increase in resources), all three of which were under both climate and herbivore control.

2.4 | Data analysis

We used fixed effects models to determine the influence of grazing treatment (grazed, ungrazed) on monthly measures of green shoot biomass (2012–2014, g/m²), soil water content (2013, 2014, %vol), leaf N concentration (2012–2014, %), and NAP (2012–2014, g m⁻² day⁻¹), and during the three sampling intervals for soil N availability (2013, mg N cm⁻² day⁻¹). The effect of herbivores on each measured plant and soil variable was modelled using grazing treatment as the single fixed effect and sample interval nested within year as random effects. This approach is similar in concept to decades of grazing studies that have examined how herbivores affect single plant or soil properties.

We also tested for direct and indirect effects of herbivores and climate on plant and soil properties within our conceptual framework (Figure 1). At each site, we performed a series of six analyses, using mixed effects models with sample interval nested in year, similar to above, to determine the best models for the six plant and soil variables (Figure 1). The Akaike information criterion for small sample sizes (AICc) was used to compare the full model that included all potential dependent variables and different versions of reduced models (Burnham & Anderson, 2002) using the model reduction protocol of Crawley (2007). We used daily average temperature and precipitation during the sampling interval to model plant and soil properties in our conceptual framework (Figure 1), with the exception that cumulative rainfall over the 7 days prior to measuring soil moisture was used to model soil moisture because it yielded the strongest correlation, compared to shorter or longer periods. Soil moisture was not included in evaluating models of soil N availability and shoot N concentration, because they were point measurements and likely did not reflect mean moisture conditions during the monthly sampling intervals. Zero values were removed from the grazed and ungrazed NAP analyses at the dry grassland to normalize the NAP data and modelled error distributions. All continuous variables were standardized by calculating the deviation from the mean divided by two standard deviations to enable relative effect size comparisons (Gelman & Hill, 2007). We report the AICc and w̄ values for the top three models for each of the soil and plant properties analysed.

Mixed effects models also were used to test if percent relative graminoid cover was a function of grazing, nested within year and spatial location. All mixed model analyses were run using the lme function of the nlme package (Pinheiro, Bates, DebRoy, & Sarkar, 2017) in R version 3.3.2 (R Developmental Core Team, 2014).

3 | RESULTS

Ungulate consumption, soil resources, plant N content, and NAP were greater at the mesic grassland compared to the dry grassland. Mean daily consumption (g m⁻² day⁻¹) was 508% greater at the mesic grassland (Figure S1), which resulted in greater grazing intensity at the mesic (71% of the NAP consumed) compared to the dry (58%) grassland. Grazed mesic grassland soil moisture (%vol) was 402%, N availability (mg N cm⁻² day⁻¹) 719%, shoot N (% NAP) 115%, green biomass (g/m²) 381% and daily NAP (g m⁻² day⁻¹) 229% greater than at the dry grassland (Figure S1). Soil moisture was likely greater at the mesic site, because of the greater soil organic matter and the finer textured soil than at the dry grassland. Soil moisture tended to be lower in the middle- and late-growing season, except at the mesic site in 2014, the wettest year of the study (Figure S1). Soil N availability increased through the growing season at the mesic grassland and remained relatively low at the dry grassland (Figure S1).

Grazers significantly reduced green shoot biomass and increased percent leaf N at both grasslands (Table 1), which resulted in lower standing biomass of more N-enriched vegetation in grazed compared to ungrazed grassland, particularly at the mesic grassland (Figure 2). At the mesic site, herbivores also increased N availability and weakly (p = .097) enhanced daily NAP (Table 1, Figure S1). Herbivores tended to facilitate daily NAP at the mesic grassland during the mid- and late-growing season in 2012 and 2013 (Figure S1). Although we found no overall effect of grazing on soil moisture, there was a significant (p < .0001) and weakly significant (p = .0577) negative relationship between the influence of herbivores on soil moisture (grazed soil moisture minus ungrazed soil moisture) and the soil moisture conditions of ungrazed plots at the mesic and dry grasslands, respectively (Figure 3). These relationships indicated that grazing reduced moisture under wet conditions and enhanced soil moisture under dry conditions at each grassland. Herbivores
increased soil moisture markedly more at the mesic grassland compared to the dry grassland (Figure 3).

Percent graminoid cover of vegetation was 34% (±4% SE) and 39% (±6% SE) in ungrazed and grazed plots, respectively, at the mesic grassland and 60% (±6%) and 55% (±7% SE) in the ungrazed and grazed

### TABLE 1 The effect of the grazing treatment, included as the single fixed variable, on daily consumption rate (g m\(^{-2}\) day\(^{-1}\)), shoot biomass (g/m\(^2\)), shoot N content (%), daily NAP (NAP\(_{\text{day}}\), g m\(^{-2}\) day\(^{-1}\), log\(_{10}\)-transformed soil moisture (\%vol), and soil N availability (mg N cm\(^{-2}\) membrane day\(^{-1}\)) at the dry and mesic grasslands

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mesic t-value</th>
<th>Mesic p</th>
<th>Dry t-value</th>
<th>Dry p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Green shoot biomass</td>
<td>-17.43</td>
<td>&lt;.001</td>
<td>-13.30</td>
<td>&lt;.001</td>
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<tr>
<td>Soil log(_{10}) (moist)</td>
<td>1.49</td>
<td>.142</td>
<td>1.23</td>
<td>.221</td>
</tr>
<tr>
<td>Soil N availability</td>
<td>3.28</td>
<td>.011</td>
<td>0.56</td>
<td>.581</td>
</tr>
<tr>
<td>Shoot N</td>
<td>6.33</td>
<td>&lt;.001</td>
<td>6.95</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>NAP(_{\text{day}})</td>
<td>1.68</td>
<td>.097</td>
<td>-0.84</td>
<td>.404</td>
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</table>

**FIGURE 2** The relationship between shoot biomass (g/m\(^2\)) and shoot N content (%) at the (a) mesic and (b) dry grasslands in YNP plots, respectively, at the dry grassland. In both grasslands, percent graminoid cover was unaffected by grazing (mesic, p = .49; dry, p = .78).

### 3.1 Model results

#### 3.1.1 Mesic grassland

Herbivory was the only factor included in the model of green biomass (see Table 1). Soil moisture at the end of the sample interval was a positive function of the soil moisture at the beginning of the interval, the amount of recent (seven-day) rainfall, and negatively related to mean daily temperature and green biomass during the interval (Table 2). Because grazers reduced green biomass, they indirectly enhanced soil moisture status which, based on Figure 3, occurred when soils were relatively dry.

Soil N availability was primarily a positive function of the main effect of grazing and secondarily a positive influence of average daily temperature and a negative interaction between grazing and green biomass (Table 2); the latter reflecting grazers enhancing soil N availability when green biomass was high. Shoot N was a positive function of soil N availability. Grazers did not directly influence shoot N content (Table 2).

We modelled NAP in ungrazed and grazed grassland separately to assess how herbivory may change controls on primary production. The single variable included in the best model of NAP for ungrazed grassland was mean shoot N concentration (%) during the sampling interval (Table 2). A bivariate plot of NAP and shoot N content explained 57% of the variation in NAP in ungrazed grassland (Figure 4a). In contrast, NAP in grazed grassland was a negative function of the initial green biomass, i.e., the green biomass at the beginning of the sample interval (Table 2). The negative influence of biomass on NAP was a surprise (we
had hypothesized that NAP would be positively related to the initial biomass and likely reflected the linkages among high biomass, high transpiration and moisture stress.

### 3.1.2 Dry grassland

Similar to the mesic grassland, grazing had a negative effect on, and was the only factor included in the model of, green biomass in dry grassland (see Table 1). Soil moisture was a function of recent precipitation (positive), and its interaction with average daily temperature (negative) (Table 2). Green biomass was an additional term in an alternative, but less likely model of soil moisture (Table S1). Soil N availability was a negative function of average daily temperature (Table 2). Shoot N content was enhanced primarily by grazing and secondarily by the amount of recent precipitation (Table 2). Daily NAP in ungrazed grassland was a function of shoot N content (Table 2), illustrated by the bivariate plot of NAP and shoot N content (Figure 4b) explaining 52% of the variation in NAP. Daily NAP in grazed grassland was negatively affected by average daily temperature and positively influenced by the soil moisture status at the beginning of the sampling interval (Table 2). A second less likely model included shoot N concentration in addition to average temperature and the initial soil moisture status (see Table S1).

### 4 DISCUSSION

Plant production in northern temperate grasslands, like YNP, is principally limited by the availability of soil moisture and N (Fay et al., 2015; Frank, 2008; Harpole & Tilman, 2007; Hooper & Johnson, 1999; Kirchner, 1977; Parton et al., 1988; Risser, 1985). Temporal variation in climate (i.e., precipitation, temperature) influences soil moisture directly and indirectly affects soil N availability by regulating microbial activity. Decades of grazing studies have documented that herbivores also influence those resources. Herbivores can increase soil moisture by reducing transpirational surface area (McNaughton, 1984; Wallace, 1990) and enhance soil N availability by adding urine and dung to the soil and stimulating N mineralization rates in rhizospheres of grazed plants (Chen et al., 2017; Frank & Groffman, 1998; Hamilton & Frank, 2001; Hamilton et al., 2008). In this study, when we examined the effects of herbivores on targeted plant or soil properties, similar to many previous studies, we found that grazers reduced shoot biomass and increased shoot N content at both grasslands (Table 1), and increased soil N availability and weakly facilitated daily NAP at the mesic grassland. We also found that herbivores increased soil moisture under dry conditions and reduced moisture when soils were wet (Figure 3); the latter likely due to trampling reducing the volume of pore space in the soil.

However, our principle goal of this investigation was to determine how the effects of climate and herbivores were propagated along pathways that regulate resources (i.e., soil moisture and N) that control NAP. We found that by enhancing the N content of shoots, herbivores reduced the influence of N on NAP at both grasslands (Table 2). In ungrazed grassland, monthly measures of NAP were a function of the single factor shoot N content (Figure 4). In contrast, NAP of grazed grassland was related to factors associated with soil moisture content (Table 2, Figure 5). At the mesic grassland, NAP was negatively associated with the initial shoot biomass, which was counter to

<table>
<thead>
<tr>
<th>Site</th>
<th>Dependent variable</th>
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<tr>
<td></td>
<td>Independent variables</td>
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<tr>
<td>Mesic</td>
<td>Log_{10} soil moisture</td>
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<td>Log_{10} soil N availability</td>
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<td>Shoot N</td>
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<tr>
<td></td>
<td>Daily NAP_{ungrazed} cubic transformed</td>
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<td></td>
<td>Log daily NAP_{grazed}</td>
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| Dry        | Log_{10} soil moisture | PPT_{7 day} | 0.025 | 1.94  | .056 |
|            |                     | PPT_{7 day}:Temp | -0.002 | -2.04  | .044 |
|            | Log_{10} soil N availability | Temp_{daily avg} | -0.088 | -6.54  | <.001 |
|            | Shoot N | Grazing treatment | 0.152 | 6.82  | <.001 |
|            | Daily NAP_{ungrazed} | Log shoot N | 1.45 | 2.46  | <.049 |
|            | Daily NAP_{grazed} | Temp_{daily avg} | -0.226 | -3.39  | <.015 |
|            |                     | Initial moisture | 0.057 | 1.26  | .221 |

**TABLE 2** Standardized parameters, t-values, and probabilities for factors included in the most likely models explaining variation in soil moisture, soil N availability, and daily net above-ground production (NAP) in grazed and ungrazed grassland at the mesic and dry sites.
our original hypothesis that NAP should be positively related to the amount of plant material available to assimilate carbon. Instead the negative relationship of NAP with biomass was likely due to greater biomass, and concomitantly, greater transpiration rates causing greater soil conditions that reduced NAP. Together, the reduction in plant biomass by herbivores and the negative effect of plant biomass on NAP indicated a positive feedback by grazers on NAP via ameliorating soil moisture conditions (Figure 5a), particularly during relatively dry periods (Figure 3). At the dry grassland, NAP of grazed grassland was a function of two climate variables affecting soil moisture, (1) mean daily temperature and (2) soil moisture at the beginning of the sampling interval (Figure 5b).

The mechanistic underpinnings of how grazers increased shoot N differed between the two grasslands. At the mesic grassland, a combination of climatic factors and herbivore effects were propagated along a pathway of linked biogeochemical processes that regulated shoot N content. Leaf N content was determined by soil N availability. Soil N availability, in turn, was strongly controlled by (1) a positive direct effect of herbivores, likely due to dung and urine deposited on the soil and/or the facilitating effects of herbivory on rhizospheric N processes (Chen et al., 2017; Frank & Groffman, 1998; Hamilton & Frank, 2001; Hamilton et al., 2008), (2) an indirect positive influence that grazers had on N availability by reducing green biomass, and presumably, enhancing soil moisture, and (3) the stimulating effect of temperature (Figure 5a). At the dry grassland, leaf N content was positively affected by grazing and recent precipitation (Figure 5b). Defoliation has been shown to stimulate tillering in a number of grass genera (Dalglish & Hartnett, 2009; Hernández Garay, Matthew, & Hodgson, 1997; Matthew, Assuero, Black, & Sackville Hamilton, 2000; Olsen & Richards, 1988). Thus, grazers may have directly increased leaf N content at the dry grassland by stimulating tillering and the production of young relatively N-enriched tissue by grazed grasses. Grazers also can enhance leaf N content by increasing the abundance of more N-enriched plant species. We found no evidence that the abundance of dicots, which are more N-enriched than graminoids in YNP (Frank, 2006), was increased by grazing. However, it is possible that herbivores may have promoted sub-groups of dicots and/or graminoids with relatively high leaf N content. Certainly the effect of a grazer-induced shifts in plant species composition on shoot N content and other plant and soil variables needs further study.

The only other measured variable that grazers affected at the dry grassland was green biomass. Unlike at the mesic grassland, standing green biomass had no influence on soil moisture in the most likely mixed effects model for the dry grassland (Table 2, Figure 5b), probably due to less canopy biomass and therefore a lower capacity for vegetation at the dry grassland to influence soil moisture conditions. A second possible, but less likely model for soil moisture at the dry grassland included green biomass (Table S1), suggesting that biomass had a weak effect on soil moisture. The greater impact of green biomass on soil moisture at the mesic grassland revealed in the models is consistent with grazing enhancing soil moisture by as much as 10% at the mesic compared to only 2% at the dry grassland during the dry portion of the growing season (Figure 3). Controls on the biogeochemistry of grazed dry grassland vs. grazed mesic grassland also differed by the amount that climate and herbivores controlled soil moisture and N availability (Figure 5). Greater climatic control at the dry site is not surprising given the lower moisture levels and thus greater moisture limitation at that grassland compared to the mesic grassland.

The reduction in N limitation of plant growth by grazers suggests that herbivores strengthen moisture limitation of YNP grassland NAP. Herbivores have been shown to enrich shoot N content in a wide range of grassland types such as North American shortgrass plains (Milchunas, Varnamkhasti, Lauenroth, & Goetz, 1995), mixed grass plains (Holland & Detling, 1990), tallgrass prairie (Turner, Seastedt, & Dyer, 1993) and tropical savanna (Cootee, Stock, & Craine, 2011; McNaughton, 1984). Consequently, herbivore amelioration of N-limitation, and a concomitant increase in moisture—limitation of NAP may be a general response of grasslands to grazing. Knapp and Smith (2001) used long-term records from arctic, desert, old-field, forest and ungrazed grassland LTER sites to examine the link between variation (CV) in annual precipitation and
NAP among different types of ecosystems. They found that grasslands exhibited the highest CV in annual NAP, which was a function of grasslands having a combination of intermediate levels (relative to other ecosystem types) of moisture limitation of NAP, variation in annual precipitation, and meristem density, which they reasoned was associated with the capacity of a system to respond to variation in precipitation. Based on results from YNP, we would expect that grazing would further enhance the responsiveness of grassland NAP to climatic variation.

An important caveat of this study is that we did not measure other minerals aside from N. Although grassland production can be limited by other nutrients in addition to N, particularly phosphorus (P). In an analysis of the effects of fertilization on 42 grasslands around the world, Fay et al. (2015) found N limitation was greatest among temperate grasslands such as YNP. Our results indicate that the strong N limitation in YNP is relaxed by grazing; but also leaves unanswered how grazer facilitation of soil and plant N processes drives NAP limitation by the availability of other nutrients.

The goal of this study was to develop a better mechanistic understanding of how herbivores shift resources controlling NAP of grasslands in YNP. Our findings indicated that climatic factors (e.g., moisture, temperature) primarily drove processes at a dry grassland, and greater moisture conditions at a mesic grassland triggered more positive direct and indirect effects of grazers on plants and soils. Regulation of primary production at both grasslands shifted from N-limitation under no grazing to moisture-limitation, and potentially greater limitation by other soil nutrients, under grazing. These results show how consumer control of YNP grassland production is driven by herbivores manipulating bottom-up mechanisms that regulate plant growth. Findings indicating that grazers strengthen climatic control of NAP, suggest that consumers may increase grassland sensitivity to climate change.

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AUTHORS’ CONTRIBUTIONS

D.A.F., R.L.W., E.W.H. and P.J.W. designed the experiment and collected data and J.D.F. helped perform analyses. D.A.F. drafted the paper and all authors contributed substantially to revisions.

DATA ACCESSIBILITY


SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.